

## CLAIMS

1. A method of measuring concentrations of first to mth (where m is an integer of 2 or more) fluorescent dyes contained in a target sample, using an imaging device having first to kth (where k is an integer of 2 or more) different detection wavelength bands, portions of the detection wavelength bands overlapping any adjacent bands, comprising:

preparing first to mth reference samples each containing only one of the first to mth fluorescent dyes respectively at predetermined unit concentrations, and acquiring a measured intensity of fluorescence emitted from each reference sample in each detection wavelength band;

taking a fluorescence image of the target sample in each detection wavelength band using the imaging device; and

executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to mth fluorescent dyes at a site in the target sample,

[Formula 24]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J^T \cdot J)^{-1} \cdot J^T \cdot \begin{bmatrix} O_1 \\ O_2 \\ \vdots \\ O_k \end{bmatrix}, \quad J = \begin{bmatrix} J_{11} & J_{12} & \cdots & J_{1m} \\ J_{21} & J_{22} & \cdots & J_{2m} \\ \vdots & \vdots & & \vdots \\ J_{k1} & J_{k2} & \cdots & J_{km} \end{bmatrix}$$

where  $O_1 - O_k$  represent values of pixels in the fluorescence images of the target sample taken in the first to kth detection wavelength bands, the pixels corresponding to the site,  $J$  is a  $k \times m$  matrix, and a component  $J_{ij}$  in the  $i$ th row and  $j$ th column (where  $i$  is any integer from 1 to  $k$ , and  $j$  is any integer from 1 to  $m$ ) in  $J$  is the measured intensity in the  $i$ th detection wavelength band of the

fluorescence emitted from the  $j$ th reference sample.

2. A method according to Claim 1, wherein the imaging device includes a multiband camera having the first to  $n$ th detection wavelength bands,

5 wherein the acquiring a measured intensity of fluorescence emitted from each reference sample in each detection wavelength band includes taking the fluorescence image of each reference sample in each detection wavelength band using the multiband camera and acquiring a value of a pixel from each fluorescence image, the pixel representing a  
10 site emitting the fluorescence in each reference sample, and

wherein the calculation of the concentrations  $c_1 - c_m$  of the first to  $m$ th fluorescent dyes includes using the value of the pixel acquired from the fluorescence image of the  $j$ th reference sample taken in the  $i$ th detection wavelength band as the component  $J_{ji}$  in the matrix  $\mathbf{J}$ .

15           3. A method according to Claim 1, wherein the imaging device includes a multiband camera having the first to kth detection wavelength bands, and

wherein the acquiring a measured intensity of fluorescence emitted from each reference sample in each detection wavelength band includes measuring spectral intensities of the fluorescence emitted from each reference sample using a spectrometer, and calculating the measured intensity in each detection wavelength band of the fluorescence emitted from each reference sample, using the spectral intensities and a sensitivity characteristic of the multiband camera for each detection wavelength band.

4. A method of measuring concentrations of first to mth

(where m is an integer of 2 or more) fluorescent dyes contained in a target sample using an imaging device having first to kth (where k is an integer of 2 or more) different detection wavelength bands, portions of the detection wavelength bands overlapping any adjacent bands, and the  
 5 imaging device having first to qth (where q is an integer of 2 or more) sensitivity modes for setting different sensitivity characteristics of the imaging device, comprising:

preparing first to mth reference samples each containing only one of the first to mth fluorescent dyes respectively at predetermined  
 10 unit concentrations, and acquiring a measured intensity of fluorescence emitted from each reference sample in each detection wavelength band and in each sensitivity mode;

taking a fluorescence image of the target sample in each detection wavelength band and in each sensitivity mode using the  
 15 imaging device; and

executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to mth fluorescent dyes at a site in the target sample,

[Formula 25]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J_1^T \cdot J_1)^{-1} \cdot J_1^T \cdot \begin{bmatrix} P_1 \\ P_2 \\ \vdots \\ P_q \end{bmatrix}, \quad P_v = \begin{bmatrix} P_{1v} \\ P_{2v} \\ \vdots \\ P_{qv} \end{bmatrix}$$

$$J_1 = \begin{bmatrix} L_{11} & L_{12} & \cdots & L_{1m} \\ L_{21} & L_{22} & \cdots & L_{2m} \\ \vdots & \vdots & & \vdots \\ L_{q1} & L_{q2} & \cdots & L_{qm} \end{bmatrix}, \quad L_{vj} = \begin{bmatrix} L_{1vj} \\ L_{2vj} \\ \vdots \\ L_{kvj} \end{bmatrix}$$

where  $P_v$  (where  $v$  is any integer from 1 to  $q$ ) is a  $k \times 1$  matrix, a component  $P_{iv}$  in the  $i$ th row (where  $i$  is any integer from 1 to  $k$ ) in  $P_v$  is a value of a pixel in the fluorescence image of the target sample taken in the  $i$ th detection wavelength band and in the  $v$ th sensitivity mode using the imaging device, the pixel corresponding to the site,  $J_1$  a  $(k \cdot q) \times m$  matrix, and a component  $L_{ijv}$  in the  $i$ th row in a component matrix  $L_{vj}$  (where  $j$  is any integer from 1 to  $m$ ) in  $J_1$  the measured intensity in the  $i$ th detection wavelength band and in the  $v$ th sensitivity mode of the fluorescence emitted from the  $j$ th reference sample.

5. A method of measuring concentrations of first to  $m$ th (where  $m$  is an integer of 2 or more) fluorescent dyes contained in a target sample using an imaging device having first to  $k$ th (where  $k$  is an integer of 2 or more) different detection wavelength bands, portions of the detection wavelength bands overlapping any adjacent portions, comprising:

preparing first to  $m$ th reference samples, each reference sample containing only one of the first to  $m$ th fluorescent dyes respectively at predetermined unit concentrations, illuminating the first to  $m$ th reference samples with each of first to  $r$ th (where  $r$  is an integer of 2 or more) excitation beams having different wavelength spectra for exciting all the first to  $m$ th fluorescent dyes, and acquiring a measured intensity in each detection wavelength band of fluorescence emitted from each reference sample;

illuminating the target sample with each excitation beam and taking a fluorescence image of the target sample in each detection wavelength band using the imaging device; and

executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to mth fluorescent dyes at a site in the target sample,

[Formula 25]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J_2^T \cdot J_2)^{-1} \cdot J_2^T \cdot \begin{bmatrix} Q_1 \\ Q_2 \\ \vdots \\ Q_r \end{bmatrix}, \quad Q_u = \begin{bmatrix} Q_{1u} \\ Q_{2u} \\ \vdots \\ Q_{ku} \end{bmatrix}$$

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$$J_2 = \begin{bmatrix} T_{11} & T_{12} & \cdots & T_{1m} \\ T_{21} & T_{22} & \cdots & T_{2m} \\ \vdots & \vdots & \ddots & \vdots \\ T_{r1} & T_{r2} & \cdots & T_{rm} \end{bmatrix}, \quad T_{uj} = \begin{bmatrix} T_{1uj} \\ T_{2uj} \\ \vdots \\ T_{kuj} \end{bmatrix}$$

where  $Q_u$  (where  $u$  is any integer from 1 to  $r$ ) is a  $k \times 1$  matrix, a component  $Q_{iu}$  in the  $i$ th row (where  $i$  is any integer from 1 to  $k$ ) in  $Q_u$  a value of a pixel in the fluorescence image of the target sample taken in the  $i$ th detection wavelength band upon illuminating the target sample with the  $u$ th excitation beam, the pixel corresponding to the site,  $J_2$  a  $(k \cdot r) \times m$  matrix, and a component  $T_{ij}$  in the  $i$ th row of a component matrix  $T_{uj}$  (where  $j$  is any integer from 1 to  $m$ ) in  $J_2$  the measured intensity in the  $i$ th detection wavelength band of the fluorescence emitted from the  $j$ th reference sample upon illuminating the  $j$ th reference sample with the  $u$ th excitation beam.

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6. A method according to any one of Claims 1 to 5, wherein the imaging device includes one or more imaging devices for taking the fluorescence images of the target sample in the first to  $k$ th detection wavelength bands to generate first to  $k$ th image signals, and an arithmetic circuit to which the first to  $k$ th image signals are fed,

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wherein the calculation of the concentrations  $c_1 - c_m$  of the first to mth fluorescent dyes includes a process in which the arithmetic circuit executes the operation using the first to kth image signals,

the method further comprising: causing the arithmetic circuit to calculate the concentrations  $c_1 - c_m$  at a plurality of sites in the target sample and to generate first to mth image signals indicating concentration distributions of the first to mth fluorescent dyes.

7. A system for measuring concentrations of first to mth (where m is an integer of 2 or more) fluorescent dyes contained in a target sample, comprising:

a photodetector for detecting fluorescence emitted from each of first to mth reference samples each containing only one of the first to mth fluorescent dyes respectively at predetermined unit concentrations, and for measuring an intensity of the fluorescence;

an imaging device having first to kth (where k is an integer of 2 or more) different detection wavelength bands and configured to take a fluorescence image of the target sample in each detection wavelength band, portions of the detection wavelength bands overlapping any adjacent bands; and

an arithmetic device for executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to mth fluorescent dyes at a site in the target sample,

[Formula 27]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J^T \cdot J)^{-1} \cdot J^T \cdot \begin{bmatrix} O_1 \\ O_2 \\ \vdots \\ O_k \end{bmatrix}, \quad J = \begin{bmatrix} J_{11} & J_{12} & \cdots & J_{1m} \\ J_{21} & J_{22} & \cdots & J_{2m} \\ \vdots & \vdots & & \vdots \\ J_{k1} & J_{k2} & \cdots & J_{km} \end{bmatrix}$$

where  $O_1 - O_k$  are values of pixels in the fluorescence images of the target sample taken in the first to kth detection wavelength bands, the pixels corresponding to the site,  $J$  is a kern matrix, and a component  $J_{ij}$  in the  $i$ th row and  $j$ th column (where  $i$  is any integer from 1 to  $k$ , and  $j$  any integer from 1 to  $m$ ) in  $J$  the intensity in the  $i$ th detection wavelength band of the fluorescence emitted from the  $j$ th reference sample, measured by the photodetector.

8. A system according to Claim 7, including a multiband camera having the first to kth detection wavelength bands as the photodetector and the imaging device,

wherein the photodetector takes the fluorescence image of each reference sample in each detection wavelength band, and acquires a value of a pixel representing a site emitting the fluorescence in each reference sample, from each fluorescence image, and

wherein the arithmetic device uses a value of the pixel acquired from the fluorescence image of the  $j$ th reference sample taken in the  $i$ th detection wavelength band as the component  $J_{ij}$  of the matrix  $J$ .

9. A system according to Claim 7, wherein the photodetector includes a spectrometer for measuring spectral intensities of the fluorescence emitted from each reference sample,

wherein the imaging device includes a multiband camera having the first to kth detection wavelength bands, and

wherein the arithmetic device calculates an intensity in each detection wavelength band of the fluorescence emitted from each reference sample, using the spectral intensities and a sensitivity characteristic of the multiband camera for each detection wavelength

band, and uses the calculated intensities as components of the matrix  $J$ .

10. A system for measuring concentrations of first to  $m$ th (where  $m$  is an integer of 2 or more) fluorescent dyes contained in a target sample, comprising:

5 a photodetector for detecting fluorescence emitted from each of first to  $m$ th reference samples each containing only one of the first to  $m$ th fluorescent dyes respectively at predetermined unit concentrations;

an imaging device having first to  $k$ th (where  $k$  is an integer of 2 or more) different detection wavelength bands and having first to  $q$ th (where  $q$  is an integer of 2 or more) sensitivity modes for setting different sensitivity characteristics of the imaging device, the imaging device taking a fluorescence image of the target sample in each detection wavelength band and in each sensitivity characteristic, portions of the detection wavelength bands overlapping any adjacent bands; and

15 an arithmetic device for executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to  $m$ th fluorescent dyes at a site in the target sample,

[Formula 28]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J_1^T \cdot J_1)^{-1} \cdot J_1^T \cdot \begin{bmatrix} P_1 \\ P_2 \\ \vdots \\ P_q \end{bmatrix}, \quad P_v = \begin{bmatrix} P_{1v} \\ P_{2v} \\ \vdots \\ P_{kv} \end{bmatrix}$$

$$J_1 = \begin{bmatrix} L_{11} & L_{12} & \cdots & L_{1m} \\ L_{21} & L_{22} & \cdots & L_{2m} \\ \vdots & \vdots & & \vdots \\ L_{q1} & L_{q2} & \cdots & L_{qm} \end{bmatrix}, \quad L_{vj} = \begin{bmatrix} L_{1vj} \\ L_{2vj} \\ \vdots \\ L_{kvj} \end{bmatrix}$$



where  $P_v$  (where  $v$  is any integer from 1 to  $q$ ) is a  $k \times 1$  matrix, a component  $P_{iv}$  in the  $i$ th row (where  $i$  is any integer from 1 to  $k$ ) in  $P_v$  a value of a pixel in the fluorescence image of the target sample taken in the  $i$ th detection wavelength band and in the  $v$ th sensitivity mode, the pixel corresponding to the site,  $J_1$  a  $(k \cdot q) \times m$  matrix, and a component  $L_{ij}$  in the  $i$ th row of a component matrix  $L_j$  (where  $j$  is any integer from 1 to  $m$ ) in  $J_1$  the measured intensity in the  $i$ th detection wavelength band and in the  $v$ th sensitivity mode of the fluorescence emitted from the  $j$ th reference sample.

11. A system for measuring concentrations of first to  $m$ th (where  $m$  is an integer of 2 or more) fluorescent dyes contained in a target sample, comprising:

a light source for generating first to  $r$ th (where  $r$  is an integer of 2 or more) excitation beams having different wavelength spectra for exciting all the first to  $m$ th fluorescent dyes;

a photodetector for measuring an intensity of fluorescence emitted from each of first to  $m$ th reference samples upon illuminating each reference sample with each excitation beam, each reference sample containing only one of the first to  $m$ th fluorescent dyes respectively at predetermined unit concentrations;

an imaging device having first to  $k$ th (where  $k$  is an integer of 2 or more) different detection wavelength bands and configured to take a fluorescence image of the target sample in each detection wavelength band upon illuminating the target sample with each excitation beam, portions of the detection wavelength bands overlapping any adjacent bands; and

an arithmetic device for executing an operation represented by a formula below, to calculate concentrations  $c_1 - c_m$  of the first to  $m$ th fluorescent dyes at a site in the target sample,

[Formula 25]

$$\begin{bmatrix} c_1 \\ c_2 \\ \vdots \\ c_m \end{bmatrix} = (J_2^T \cdot J_2)^{-1} \cdot J_2^T \cdot \begin{bmatrix} Q_1 \\ Q_2 \\ \vdots \\ Q_r \end{bmatrix}, \quad Q_u = \begin{bmatrix} Q_{1u} \\ Q_{2u} \\ \vdots \\ Q_{ku} \end{bmatrix}$$

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$$J_2 = \begin{bmatrix} T_{11} & T_{12} & \cdots & T_{1m} \\ T_{21} & T_{22} & \cdots & T_{2m} \\ \vdots & \vdots & & \vdots \\ T_{r1} & T_{r2} & \cdots & T_{rm} \end{bmatrix}, \quad T_{uj} = \begin{bmatrix} T_{1uj} \\ T_{2uj} \\ \vdots \\ T_{kuj} \end{bmatrix}$$

where  $Q_u$  (where  $u$  is any integer from 1 to  $r$ ) is a  $k \times 1$  matrix, a component  $Q_{iu}$  in the  $i$ th row (where  $i$  is any integer from 1 to  $k$ ) in  $Q_u$  a value of a pixel in the fluorescence image of the target sample taken in the  $i$ th detection wavelength band upon illuminating the target sample with the  $u$ th excitation beam, the pixel corresponding to the site,  $J_2$  a  $(k \cdot r) \times m$  matrix, and a component  $T_{ij}$  in the  $i$ th row of a component matrix  $T_{uj}$  (where  $j$  is any integer from 1 to  $m$ ) in  $J_2$  the measured intensity of the fluorescence in the  $i$ th detection wavelength band upon illuminating the  $j$ th reference sample with the  $u$ th excitation beam.

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12. A system according to any one of Claims 7 to 11, wherein the imaging device includes one or more imaging devices for taking the fluorescence images of the target sample in the first to  $k$ th detection wavelength bands to generate first to  $k$ th image signals, and an arithmetic circuit as the arithmetic device to which the first to  $k$ th image signals are fed, and

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wherein the arithmetic circuit executes the operation using the first to kth image signals to calculate the concentrations  $c_1 - c_m$  at a plurality of sites of the target sample, and generates first to mth image signals indicating concentration distributions of the first to mth fluorescent dyes.

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